PB80-216856

Thiophanate-Methyl
Position Document 1

(U.S.) Environmental Protection Agency
Arlington, VA

1 Dec 78

U.S. Department of Commerce
National Technical Information Service
NTIS

50272-101

REPORT DOCUMENTATION PAGE	1. REPORT NO. EPA/SPRD-80/01	2.	3. Recipient's Accession No. PB 80 216856
4. Title and Subtitle: Thiophanate-Methyl: Position Document #1			5. Report Date: 12/1/78
			6.
7. Author(s): Esther Saito			8. Performing Organization Rept. No.
9. Performing Organization Name and Address: Special Pesticide Review Division, Environmental Protection Agency, Crystal Mall #2, Arlington, VA			10. Project/Task/Work Unit No.
			11. Contract(C) or Grant(G) No. (C) (G)
12. Sponsoring Organization Name and Address: Environmental Protection Agency, 401 M St. S.W, Washington, D.C. 20460			13. Type of Report & Period Covered
			14.

15. Supplementary Notes

16. Abstract (Limit: 200 words)

Preliminary Risk Assessment: Examination of possible unreasonable risks associated with uses of pesticide and a gathering of all available information to determine whether or not this or any other risk does exist. Initiates literature search and evaluates risk data. Limited information on exposure to forecast extent ot risk.

17. Document Analysis a. Descriptors

0606,0703

b. Identifiers/Open-Ended Terms

0606

c. COSATI Field/Group

18. Availability Statement: Release Unlimited	19. Security Class (This Report): Unclassified	21. No. of Pages
	20. Security Class (This Page): Unclassified	22. Price

(See ANSI-Z39.18) See Instructions on Reverse

OPTIONAL FORM 272 (4-77)
(Formerly NTIS-35)
Department of Commerce

EPA/SPRD - 80/01

PB80-216856

Thiophanate-methyl: Position Document 1

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NATIONAL TECHNICAL
INFORMATION SERVICE
U.S. DEPARTMENT OF COMMERCE
SPRINGFIELD, VA. 22161

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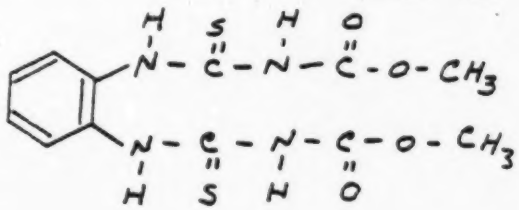
I. Background

A. Chemistry

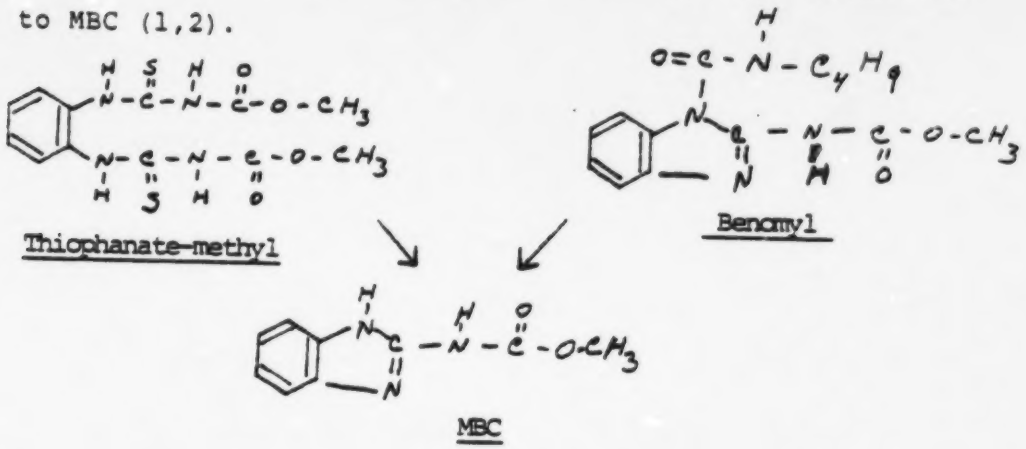
1. General Chemistry

Thiophanate-methyl is the common name for the chemical 1.2-~~C~~(3-methoxycarbonyl-2-thioureido)benzene. The accepted chemical name used on pesticide labels is dimethyl [(1,2-phenylene) bis(iminocarbonothioyl)] bis[carbamate]; it is sold under the trade names Topsin M^{R}, Cercobin M^{R} and Thiophhanate M. Thiophanate-methyl has the molecular formula $C_{12}H_{14}N_4O_4S_2$ and a molecular weight of 342. The structural formula is:



Thiophanate-methyl, a colorless crystalline solid melts at 168°C with decomposition. Thiophanate-methyl is slightly soluble in n-hexane, hardly soluble in water and sparingly soluble in most organic solvents. The compound is stable in acidic solutions, has a slight but measureable formation of methyl 2-benzimidazole carbamate (MBC) at pH7, and is unstable in alkaline solution.

Thiophanate-methyl and benomyl both metabolize to MBC (1,2).



Thiophanate-methyl

Benomyl

MBC

MBC is a powerful fungicide that is generally regarded as the actual fungitoxic principle of benomyl (1). Vonk and Sipesteijn found a correlation between the formation of MBC and the fungitoxic activity of thiophanate-methyl (2). Thiophanate-methyl was completely converted to MBC in 0.066 M phosphate buffer, pH7, at 70°C in 26 hours (2).

2. Residue Chemistry

a. Soil

In moist soil thiophanate-methyl was degraded rapidly; 80 to 90% had disappeared from Towner sand and Fargo clay soils five days after application (3). Thirty days after application of thiophanate-methyl to air-dried soil 10 to 20% thiophanate-methyl could be detected. The principal metabolite found in the soils was MBC. (3)

b. Animals

Noguchi and coworkers (4) and Fujino and coworkers (5), cited in the World Health Organization Technical Report Series (1974), reported that feeding ^{14}C- or ^{35}S-labelled thiophanate-methyl to rats, mice, and dogs showed 80 to 100% recovery in feces and urine within 96 hours. MBC has been identified as the major metabolite in both mice and dogs in studies submitted to EPA by Noguchi and coworkers (33).

Fujino and coworkers report unmetabolized thiophanate-methyl as the major part of the fecal excretion. The minor part consisted of 4-hydroxy-thiophanate-methyl (4-OH-TM) and dimethyl-4.4'-O-phenylenebisallophanate (FH-432). MBC and 5-hydroxy-MBC (5-OH-MBC) were observed but the authors questioned if they were metabolites or were produced during the analytical procedures (5).

Kosaka and co-workers, cited in the World Health Organization Technical Report Series (1976), reported that 5-hydroxy methyl benzimidazole was the main metabolite in the urine of female rats, which had been administered ^{14}C-labelled thiophanate-methyl. Thiophanate-methyl, 5-hydroxy methyl benzimidazole, 4-hydroxy-dimethyl-4,4'-O-phenylene bis-allophanate were identified as metabolites in the feces (6).

c. Plants

Thiophanate-methyl was converted to MBC after uptake through roots and after foliar treatment of French bean seedlings (4) Buchenauer and coworkers (7) found that the transformation is to a great extent catalyzed by light in addition to requiring water. Thiophanate-methyl residues in cotton leaves were transformed to MBC; after four days in sunlight 42% were transformed as opposed to 6% when the plants were kept in dim light.

B. Uses and Production

Thiophanate-methyl was first registered for use as a fungicide in Japan in May 1971. Great Britan granted provisional clearance for a number of preharvest treatments on food crops and ornamentals and post-harvest dipping of apples in 1973 (8). Thiophanate-methyl is only registered for use on turf and ornamentals in the U.S.A.

Pennwalt is the only registered producer of technical grade thiophanate-methyl in the United States. Under Section 7(c) of the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) manufacturers and formulators are required to submit information on production, sales, and distribution. Under Section 10, this information is considered confidential and cannot be released to the public, but it is made available to the Administrator in a confidential appendix to this report (9).

II. Regulatory History

The first registration for a product containing thiophanate-methyl was granted January 16, 1973, to Pennwalt for technical grade product (EPA Registration Number 4581-280). In July and August 1973, three companies registered products containing 50 to 70% thiophanate-methyl and formulated as wettable powders for use on turf. A wettable powder containing 1.15% thiophanate-methyl was registered for use on turf in February 1974. A product which contains 25% thiophanate methyl and 15% 5-ethoxy-3-(trichloromethyl)-1,2, 4-thiadizole was registered for use on flowers and ornamentals in July 1974.

Tolerances were established, under 40 CFR Section 180.371 for residues on bananas (2 ppm of which only 0.2 ppm may be present in the pulp) on February 3, 1977; tolerances for residues on stone fruits (15 ppm) and strawberries (5 ppm were added on March 17, 1977.

Although no products containing thiophanate-methyl are registered for use on food crops, an application is being reviewed for use on stone fruits and strawberries.

III. Summary of Scientific Evidence to Support Rebuttable Presumption

40 CFR 162.11 (a)(3) provides that a rebuttable presumption shall arise if a pesticide's ingredients, metabolites

or degradation products meet or exceed (i) acute toxicity risk criteria relating to hazards to humans, domestic animals, or wildlife, or (ii) chronic toxicity risk criteria relating to humans and to population reductions in non-target organisms or fatality to members of endangered species.

A. Chronic Toxicity

1. Mutagenic Effects in Multitest Systems

Section 162.11 (a)(3)(ii)(A) provides that a rebuttal presumption shall arise if a pesticide's ingredients, metabolites, or degradation products induces mutagenic effects, as determined by multitest evidence.

Studies in plants (4,7) animals (33) and soil (3) have shown that thiophanate-methyl is metabolized to MBC. MBC has been shown to produce somatic chromosomal abnormalities and point (gene) mutations in microbial and mammalian test systems. Further evidence of the mutagenic effects of thiophanate-methyl and MBC has been obtained in studies of plants. MBC has also been shown to reach gonadal tissues in mammals with physiological effects; hence it has the potential of interacting with genetic material.

a. Chromosomal Effects

i. Mammalian Cells in Culture

MBC has been shown to cause mitotic delay (10), a low incidence of chromosomal breakage (10), and multi-nucleation (10, 11) in human, mouse, and hamster cells lines.

ii. Mammalian Tests in Vivo

Metaphase arrest and micronuclei formation occurred in bone marrow cells of rats treated with MBC; in addition there was a low frequency of chromosomal breakage and formation of anaphase bridges (10).

Seiler (1976) found that MBC produced dose-related mutagenic effects in the micronucleus test in mice. In addition, lagging chromosomes, anaphase bridges, unequally distributed chromosomes, and tripolar anaphases appeared in mitotic bone marrow cells of treated animals; none of these effects were noted in control cells (12). The author concluded that the main action of MBC was the inhibition of the mitotic spindle, which may predispose to nondisjunctional errors. The finding of anaphase bridges indicates that MBC can break chromosomes.

On the other hand thiophanate-methyl administered intraperitoneally (dosage range from 8 to 500 mg/kg) did not increase the frequency of dominant lethal mutations in

ICR-strain mice (13). Abnormal chromosome configuration were not observed in bone marrow or spermatogonial cells of Wistar strain albino male rats treated daily for 5 days with thiophanate-methyl by intraperitoneal injection (62.5 to 1000 mg/kg) (13).

iii. Fungi and Molds

MBC interfered with normal nuclear division in Saccharomyces cerevesiae, Ustilago maydis (12), Botrytis cineara (13), and Aspergillus nidulans (14). Interference with cell division may predispose to chromosomal nondisjunction.

b. Point Mutations

i. Bacteria

Two studies have reported that MBC and other benzimidazoles induce base-substitution mutations as detected by the Ames test without metabolic activation. Salmonella typhimurium strains his G46, TA1530, and TA1535 were used in the tests. Forward mutations were induced in Salmonella strain LT-2 by MBC; reverse mutations were induced in Escherichia coli strain WP2 uvrA which is excision deficient but not in strain WP2, which is excision proficient (17,18). Mutations appear to result from the incorporation of benzimidazoles into DNA in place of purine bases (19); it has been hypothesized that mutation occurs when the abnormal base is recognized by the cell but the defect is not repaired

The Agency staff has evaluated the methodology, protocol, and conclusions of several studies that indicate that thiophanate-methyl is highly toxic to earthworms. In studies by Stringer and Wright (22) earthworm populations were "virtually eliminated" in apple orchard plots sprayed with thiophanate-methyl. In addition, thiophanate-methyl was repellent to earthworms, apparently because it made food materials unpalatable to them. The spraying of thiophanate-methyl in orchards reduced not only the number and biomass of all earthworm species combined, but also that of each individual species (23).

Stringer and Lyons stated that earthworms are generally regarded as beneficial animals in maintaining soil fertility, but noted that it has not been rigorously proven that they are essential (23). Hopp claims that earthworm activity is not one of the three basic requirements for plant growth but is a factor which can be used to correct deficiencies in soil fertility (24). Thus, the Working Group is unable to adequately evaluate the environmental, agricultural, and economic significance of these population reductions. Comment is, therefore, especially solicited on the relevance, impact, and long-term significance of such local population reductions.

The Working Group recognizes that many other factors contribute to the size of earthworm populations, including soil composition, moisture, temperature, cultivation practices, and the use of other pesticide. Furthermore, it appears that the adverse effects on earthworms are generally limited to areas of application and do not extend significantly to adjacent areas. That is, the effects appear to be confined largely to privately owned land, the owners of which can be made aware of the likely effects on earthworms. Nonetheless, it is clear that the use of thiophanate-methyl according to current label directions can reasonably be expected to result in significant local reductions of earthworm populations. Accordingly, the Working Group concludes that a rebuttable presumption has arisen against pesticide products containing thiophanate-methyl which are registered for outdoor use.

IV. Other Relevant Adverse Effects

A. Potential Formation of N-Nitroso Compounds

Many chemicals can react with nitrites to form N-nitroso compounds (25) which as a class are recognized as animal carcinogens and mutagens (26).

Two papers (27,28) dealing with the possible formation of N-nitroso MBC and induction of lymphosarcomas were reviewed. Both papers reported that neither MBC nor sodium nitrite alone produced tumors; tumors did develop in Swiss

mice given large amounts of sodium nitrite (0.05 to 0.5% in drinking water) and administered MBC by gavage.

The fact that lymphosarcomas form in Swiss mice treated with MBC and nitrite but not in mice treated with MBC alone suggests that MBC and nitrite interact to create a stress that results in tumor production (27). Furthermore, offspring of mice treated with MBC and nitrite during gestation developed tumors, whereas offspring of animals treated with MBC alone did not (28).

Evaluations of these studies state that the data do not provide evidence for in vivo production of N-nitroso MBC (29,30). Additional data necessary in order to evaluate the hazards of N-nitroso MBC would include kinetic studies and information on the isolation of an N-nitroso MBC derivative formed in vivo, and rate of formation of N-nitroso MBC in the soil and rate of uptake into plants (30).

Although there is no evidence at present that N-nitroso compounds are formed from MBC, nitrosation can take place on either of the two nitrogen sites. Formation of N-nitroso compounds might occur after the pesticide is applied if nitrites are present. Such formation is therefore possible in soil, water, air, plants, and animals, including man (30).

The potential formation of N-nitroso MBC is a matter of concern. The Working Group recommends that the Agency solicit further information on the formation of N-nitroso compounds from MBC, the conditions necessary for formation of such compounds, and their potential as carcinogenic and mutagenic agents.

B. Teratogenic and reproductive effects

The Working Group has considered a paper on the teratogenic effects of thiophanate-methyl, which has been reviewed by Battelle Memorial Laboratories (31). Makita and co-workers administered thiophanate-methyl by gavage to ICR mice (dosage levels from 40 to 1000 mg/kg) from day 1 to day 15 of gestation (13). The only observed malformation was cleft palate which occurred with the same incidence in the control and experimental animals. The highest dosage level (1000 mg/kg) resulted in a slight reduction in the number of living fetuses with the no-effect level (500 mg/kg) appearing to represent an adequate margin of safety. However, the study should be considered inconclusive for determining teratogenic effects because apparently brain, spinal cord, heart, and urogential organs were not examined (31).

In a 3-generation reproductive study of the effect of thiophanate-methyl on the rat, Palmer and co-workers administered thiophanate methyl in the diet to CD strain (Charles River) rats (70-80 g) at levels of 0, 40, 160, and

In a 3-generation reproductive study of the effect of thiophanate-methyl on the rat, Palmer and co-workers administered thiophanate-methyl in the diet to CD strain (Charles River) rats (70-80 g) at levels of 0, 40, 160, and 640 ppm. At the 640 ppm level a slightly lower litter size from birth to weaning consistenly occurred in all generation and there was a tendency for lower litter weight from birth through lactation to weaning. Skeletal variations, which did not appear in the control animals, were observed at the three experimental dosage levels, although the anomalies were not dose dependent (32).

The Working Group recommends that the Agency solicit additional information on potential teratogenic effects of thiophanate-methyl and exposure from actual use.

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